conformational re-arrangements that promote diminished or increased ligand-affinity. Our findings give clues for understanding the molecular mechanisms used by the emerging GLTP-fold protein superfamily to selectively transfer certain lipids between membranes. [Support: Spanish Ministerio de Ciencia e Innovacion (MICINN BFU2010-17711), NIH/NIGMS GM45928 & NIH/NCI CA121493, Russian Fnd. for Basic Research 012-04-00168, Abby Rockefeller Mauze Trust, Maloris Fnd., and Hormel Fnd.].

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Nonvesicular Trafficking of Ceramide-1-Phosphate by a Lipid Transfer Protein that Regulates Eicosanoid Production

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Phosphorylated sphingolipids such as ceramide-1-phosphate (C1P) and sphingosine-1-phosphate (S1P) have emerged as key regulators of cell growth, survival, migration, and inflammation. C1P produced by ceramide kinase is an activator of group IVA cytosolic phospholipase A2α (cPLA2α), the rate-limiting releaser of arachidonic acid used for pro-inflammatory eicosanoid production. To modulate eicosanoid action and avoid the damaging effects of chronic inflammation, cells require efficient targeting, trafficking, and presentation of C1P to specific cellular sites. Vesicular trafficking is likely but nonvesicular mechanisms for C1P sensing, transfer, and presentation remain unexplored. We have identified a ubiquitously-expressed lipid transfer protein (CPTP) that can specifically transfer C1P between membranes. Crystal structures establish C1P binding via a novel surface-localized, phosphate headgroup recognition center connected to an interior hydrophobic pocket that adaptively expands to ensheath differing-length lipid chains using a cleft-like gating mechanism. The two-layered, α helically-dominated 'sandwich' topology identifies CPTP as the prototype for a new GLTP-fold subfamily. CPTP resides in the cell cytosol but associates with the trans-Golgi/TGN, nucleus, and plasma membrane. RNAi-induced CPTP depletion elevates C1P steady-state levels and alters Golgi cisternae stack morphology. The resulting C1P decrease in plasma membranes and increase in the Golgi complex stimulate cPLA2α release of arachidonic acid, triggering pro-inflammatory eicosanoid generation. [Support: NCI CA121493 (DJP & REB), NIGMS GM45928 (REB), NIGMS GM072754 (EHH), NCI CA154314 (CEC), VA Merit Award (CEC), VA Research Career Scientist Award (CEC), VA Career Devel. Award (DSW), NRS-T32/NIGMS 008695 (DSW), Spanish Ministerio de Ciencia e Innovacion BFU2010-17711 (LM), Russian Fnd. for Basic Research #12-04-00168 (JGM), Abby Rockefeller Mauze Trust (DJP), Maloris Fnd. (DJP), and Hormel Fnd. (REB); Equal contributors to the work: DKS, RKK, & DSW; Corresponding authors: REB, EHH, CEC, DJP]

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Studying Lipid Interactions of Perilipin 3/ Tip 47 using Phospholipid Monolayers

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Lipid droplets are found in all eukaryotes and are considered as dynamic cell organelles. In addition to acting as neutral lipid stores, they are also involved in various biological processes such as membrane formation, cell signaling and hormone synthesis. Many aspects of lipid droplet biogenesis and function are not yet fully understood. The basic structure of a lipid droplet consists of a neutral lipid core of triacylglycerols and cholesterol esters surrounded by a monolayer of phospholipids, free cholesterol and proteins. Perilipin3 or TIP 47 is an exchangeable PAT family protein which is associated with the surface of lipid droplets and is ubiquitously expressed. The structure of perilipin 3 consists of a PAT domain, an 11-mer repeat region, a 4-helix bundle sequence and a hydrophobic cleft region. In this study we used several N-terminal truncation mutants of human perilipin 3 (117-434AA, 152-434AA and 187-434AA) to investigate the lipid binding properties of various domains of the protein. Interactions were measured as a function of the increase of surface pressure at a lipid- buffer interphase by inserting the proteins beneath a Langmuir monolayer of phospholipids which represents the monolayer surrounding the lipid droplet. This work sheds light on the regions responsible for the localization of perilipin 3 at the surface of lipid droplets.

1546-Pos Board B276

Toward Understanding the Role of Amot80 Lipid Binding in Cellular Proliferation and Migration

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Background: Amots are adaptor proteins which coordinate signaling that controls cellular differentiation and proliferation, and their Amot coiled-coil homology (ACCH) domain is able to bind lipids with specificity which leads to membrane deformation and targets transcription factors to the nucleus. Understanding the biophysical mechanisms involved in lipid binding may provide pathways to modulate protein sorting and downstream signaling events inducing cellular differentiation, cancer cell proliferation, and migration. At this time, all work reported on signaling based on Amot expression is unable to distinguish between the role of the Amot80 and the 130 family members as they share a common ACCH domain.
Objective: The goal of this project is to specifically relate the Amot80 ACCH lipid binding with function related to cancer phenotypes.
Method: Mutations were carried forward based on lipid sedimentation, FRET, and SAXS assays against the ACCH domain of the protein. Site-directed mutagenesis was then employed to probe the specific contributions of 7 selected lysines and arginines toward lipid head-group binding in the full length protein. The polarity/scaffolding signaling effect of mutations in the Amot80 will be monitored by matrigel, accumulation/cell counting, and tritiated thymidine incorporation assays. Cell morphology will be imaged by confocal imaging, and cellular migration will be recorded by video. The effects on YAP1/2 and MAPK activation will be assessed by immunoblot analysis. The changes will then be correlated in extracellular scaffolding and migration with immunoblots and cellular staining. Likewise, effects on proliferation will be monitored by MTT assays.
Expected Results: The hypothesis of this aim is that modulation of Amot's ability to bind selective lipids will interrupt the signaling pathways leading to cellular migration, differentiation, and proliferation.
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Cation-Dependent Behavior of Cardiolipin-Containing Membranes and Implications for Respiratory Complex II Assembly and Activity

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Cardiolipin (CL) is the signature phospholipid of energy-conserving mitochondrial inner membrane. It has a uniquely dimeric structure, with a headgroup composed of two phosphatidyl moieties linked by a glycerol bridge and a hydrophobic domain of four acyl chains. Despite having two phosphodiesters, CL is proposed to be monoanionic at physiological pH due to resonance stabilization of a bicyclic acid-anion structure that traps a proton. Moreover, CL displays phase polymorphism by undergoing a lamellar to inverted hexagonal transition in the presence of divalent cations. We have investigated the influence of divalent cations on lipid bilayers containing CLs with varying headgroups and acyl chains. We analyzed global physical properties of membranes in liposomes and nanodiscs, using fluorescent probes that localize to different bilayer depths. The environment-sensitive probe Laurdan was used to detect changes in hydration level of the interfacial region. With increasing concentrations of divalent cations, we observed a CL-dependent biphasic increase in degrees of lipid packing that is profoundly reduced for CL analogs lacking the resonance-stabilized headgroup or containing the anionic lipid phosphatidylglycerol. Parallel measurements of fluorescence anisotropy using diphenylhexatriene localized in the hydrophobic bilayer interior revealed much less pronounced effects on acyl chain dynamics with identical cation titrations. Finally, based on our recent report that CL is critical for respiratory Complex II structure and function, we investigated effects of divalent cations on activity and integrity of this enzyme. We observed cation-dependent disassembly of Complex II with an attendant loss of its redox activity that shows remarkable correlation with our fluorescence-detected alterations in bilayer structure. Based on these results, we propose a model in which coordination of divalent cations by CL causes specific changes in bilayer structure that are linked with structural alterations in CL-binding protein complexes.